Azithromycin Combination Therapy for the Treatment of Uncomplicated Falciparum Malaria in Bangladesh: An Open-Label Randomized, Controlled Clinical Trial

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Background. In recent studies, the combination of azithromycin and artesunate has proven to be a promising alternative for the treatment of uncomplicated falciparum malaria.

Methods. We conducted a randomized, controlled clinical trial assessing the efficacy of azithromycin-artesunate combination therapy. The study was conducted involving 228 patients aged 8–65 years. Patients were randomized to 1 of 2 cohorts at a ratio of 2:1, receiving either azithromycin-artesunate once daily for 3 days (30 mg/kg per day of azithromycin plus 4 mg/kg per day of artesunate) or an adult dose of 80 mg of artemether plus 960 mg of lumefantrine (4 tablets Coartem or the equivalent for children weighing <35 kg) twice daily for 3 days.

Results. The 42-day cure rate by Kaplan-Meier analysis was 94.6% (95% confidence interval [CI], 89.38%–97.44%) in the azithromycin-artesunate arm and 97.0% (95% CI, 89.45%–99.40%) in the control arm. Fever clearance times and parasite clearance times did not show any differences between the 2 arms (P = .95, respectively). No serious adverse events were seen, but the percentage of patients who developed any adverse event was higher in the control group (P = .03).

Conclusions. Our data suggest that azithromycin-artesunate is an efficacious and well-tolerated treatment for patients with uncomplicated falciparum malaria in Bangladesh.

Trial Registration. ClinicalTrials.gov identifier: NCT00356005.

Malaria remains a substantial public health problem in many Asian countries, and the spread of drug resistance to commonly used antimalarials is further aggravating the situation. Bangladesh officially reports ∼400,000 clinical cases and >57,000 laboratory-confirmed malaria cases per year, with >500 deaths [1]. Thirteen of the 64 districts in the country are seriously affected by malaria, accounting for ∼99% of the country’s malaria burden. About two-thirds of the cases occur in the Chittagong Hill Tracts. Recent unpublished data from our group suggest a high prevalence of malaria in that region, with a high percentage of asymptomatic carriers.

In recent smaller studies in Thailand [2], the combination of azithromycin and artesunate has proven to be a highly efficacious and well-tolerated regimen for the treatment of uncomplicated falciparum malaria. Azithromycin is a relatively new macrolide antibiotic with good tolerability and proven activity against chloroquine-resistant Plasmodium falciparum not only in vitro [3, 4] but also in treatment and prophylaxis [5–7], as well as against Plasmodium vivax [8, 9]. However, current data indicate that relatively high doses of azithromycin are required for the treatment of falciparum malaria, and that azithromycin as a slow-acting agent needs to be combined with faster acting antimalarials.
Combination therapy for malaria is widely accepted; favorable partners for the slow-acting azithromycin are artesunate and its derivatives or quinine, which are known to more rapidly reduce the initial parasite burden. There is also extensive experience with the use of azithromycin in pregnant women and children, the populations most affected by malaria [10].

We present data from a randomized, controlled clinical trial with azithromycin combination therapy with the aim of determining the efficacy of azithromycin-artesunate as a potential alternative to currently used regimens for the treatment of uncomplicated falciparum malaria in adults and children.

PATIENTS AND METHODS

Study site and participants. The study was conducted at the Malaria Research Initiative Bandarban (MARIB) field site at the Bandarban Sadar Hospital, in Southeastern Bangladesh, a collaborative research effort of the Medical University of Vienna, the Ministry of Health and Family Welfare, and the International Centre for Diarrhoeal Disease Research, Bangladesh. Study participants were between 8 and 65 years of age and had acute symptomatic (fever or a reported history of fever within the last 48 h) Plasmodium falciparum monoinfection, with a parasite density of 100–100,000 asexual parasites per microliter of blood, as determined from the screening blood smear. Patients who had a known history of intolerance or hypersensitivity to the study drugs, who had a history of receiving antimalarial therapy in the past 4 weeks, who presented with signs and symptoms of severe malaria according to World Health Organization (WHO) criteria, or who showed laboratory evidence of significant liver or renal function abnormalities (creatinine and/or bilirubin levels of >3 mg/dL) were excluded. Females >12 years of age were required to have a negative human chorionic gonadotropin pregnancy test (hCG One Step, ACON International) and to use an acceptable method of contraception throughout the study (such as implanted, injectable, or oral contraceptive[s] with additional barrier contraception, an intrauterine device, sexual abstinence, or a vasectomized partner). Written informed consent was obtained from all study participants or their legal guardians, and the study was approved by the ethical review boards of the Medical University of Vienna and the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B).

Study design. The study design was an open-label, phase II/III, single-center, randomized, controlled clinical trial involving patients with uncomplicated P. falciparum malaria. Patients were admitted to the hospital for the duration of study drug administration or until complete fever and parasite clearance, whichever took longer. Patients were asked to return for follow-up visits on days 7, 14, 21, 28, 35, and 42 and whenever signs and symptoms consistent with malaria were reported. To limit the chances of reinfection, all patients were issued personal insecticide-treated mosquito nets.

Sample size. A sample size of 210 evaluable subjects in 2 cohorts was calculated on the basis of an expected failure rate of 10%. The sample size was calculated to allow for an estimation of failure rates within 5 percentage points with 95% confidence interval [CI] [12]. The study was not powered to prove equivalence of the treatments.

Randomization. Patients were randomly assigned in a 2:1 ratio to 1 of the 2 treatment groups by reference to a statistical series based on random sampling numbers drawn up for each patient by the study staff; the details of the series were unknown to any of the investigators or to the coordinator and were contained in a set of sealed envelopes.

Study drugs. Azithromycin (Zithromax 500 mg tablets, lots 56407003 and 66404511) was provided by Pfizer; artesunate (Artesunet, 50 mg tablets, Aexim Pharmaceuticals, lot 5081) and Coartem (Novartis, lot X0322) were purchased locally.

Patients were randomized to one of the following treatment groups: patients in the study group (cohort 1) received 1500 mg of azithromycin once daily plus 200 mg of artesunate adult dose or the equivalent for children <35 kg (30 mg/kg per day of azithromycin plus 4 mg/kg per day of artesunate) once daily for 3 days. Patients in the control group (cohort 2) received 80 mg of artemether plus 480 mg of lumefantrine (4 tablets of Coartem) or the equivalent for children below 35 kg (4 and 24 mg/kg per day) twice daily for 3 days. All antimalarial medications were administered under direct observation; artemether-lumefantrine was given with milk to improve absorption. Symptomatic treatment (with paracetamol, domperidon, and/or metoclopramid) was provided, as needed, for fever, headache, myalgias, nausea, and/or dizziness. Four patients also received omeprazole as concomitant medication during the first 3 days.

Evaluations. The findings of the physical examination, as well as any adverse events, clinical signs and symptoms, and the medication history were recorded daily until discharge and after that at every follow-up visit. Similarly, vital signs including temperature, blood pressure, pulse, and respiration rate were assessed 3 times daily until discharge and at every follow-up visit. A complete blood cell count and blood chemistry analyses were performed on the day of enrollment and day 3 or whenever clinically warranted.

Malaria blood smears were performed at enrollment and twice daily after that until 2 consecutive slides were confirmed to be negative for parasites, as well as at every follow-up visit. All parasite-positive slides after initial parasite clearance were reexamined by a second microscopist. If the findings of the 2 microscopists were discordant with respect to either species or positivity, the slides were reexamined by a third reference microscopist, whose reading was accepted as final. Microscopists...
Figure 1. Flow diagram of the progress through the phases of the trial from enrollment until data analysis.

Clinical efficacy. We screened 238 subjects; 228 were randomized and 193 patients were evaluable for primary end point analysis. Five patients, 2 in the azithromycin-artesunate arm and 3 in the control group, were lost to follow-up during the course of the trial. Twelve patients developed *P. vivax* parasitemia between days 21 and 41, 8 of them in the azithromycin-artesunate arm, and 4 in the control group. All patients who dropped out, except for a single patient, did so after complete clearing of parasites and fever. In the azithromycin-artesunate group, 128 subjects were evaluable for primary end point analysis, and 65 were evaluable in the control group (Figure 1). Both treatment groups were well matched in regard to sex, age,
Table 1. Demographic and Baseline Characteristics of Study Subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Study group&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Control group&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, no. of subjects (% children)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>107 (43.0)</td>
<td>55 (54.5)</td>
</tr>
<tr>
<td>Female</td>
<td>45 (55.5)</td>
<td>21 (57.1)</td>
</tr>
<tr>
<td>Age, years, mean ± SD</td>
<td>22 ± 13.38</td>
<td>21 ± 13.48</td>
</tr>
<tr>
<td>Weight, kg, mean ± SD</td>
<td>41.04 ± 14.56</td>
<td>38.73 ± 13.81</td>
</tr>
<tr>
<td>Height, cm, mean ± SD</td>
<td>149.38 ± 18.42</td>
<td>146.43 ± 18.73</td>
</tr>
<tr>
<td>Parasite density, asexual parasites/μL</td>
<td>9781</td>
<td>9693</td>
</tr>
</tbody>
</table>

**NOTE.** SD, standard deviation.

<sup>a</sup> Regimen: 1500 mg of azithromycin once daily plus 200 mg of artesunate for adults or 30 mg/kg per day of azithromycin plus 4 mg/kg per day of artesunate for children weighing <35 kg for 3 days.

<sup>b</sup> Regimen: 80 mg of artemether plus 960 mg of lumefantrine (4 tablets Coartem or the equivalent for children weighing below 35 kg) twice daily for 3 days.

During the course of the study, 8 patients in the azithromycin-artesunate arm developed late treatment failure, of which 3 were classified as having late clinical failure with a body temperature ≥37.5°C, whereas 5 patients had late parasitological failure. Molecular analysis suggested that 14 patients had acquired a reinfection with *P. falciparum* during the 42 days of follow-up in the azithromycin-artesunate arm. The control group was found to have 2 patients with late treatment failure, all of them late parasitological treatment failure. In addition, 4 patients in this group presented with reinfection.

The probability estimate for ACPR at day 42 (42-day cure rate) in Kaplan-Meier analysis was 94.6% (95% CI, 89.38%–97.44%) in the azithromycin-artesunate group and 97.0% (95% CI, 89.45%–99.40%) in the control group. Cure rates for days 35, 28, 21, 14, and 7 were 94.6%, 94.6%, 99.3%, 100.0%, and 100.0% in arm 1 and 97.0%, 100.0%, 100.0%, 100.0%, and 100.0% in arm 2, respectively (Figure 2). There was no difference in the 42-day cure rate between the treatment groups (*P* = .5), but the study was not powered to prove equivalence.

The mean time (± SD) until reemergence of parasites in the peripheral blood was 31.5 ± 5.0 days. All treatment failures except for one in the azithromycin-artesunate arm occurred during the fourth week of follow-up (26.9 ± 2.5 days), whereas patients in the control group developed treatment failure ~1 week later, on day 35 (*P* = .04). Reinfections occurred after 33.8 ± 5.3 and 30.7 ± 2.5 days, respectively, in the azithromycin-artesunate group and the control group.

Enrollment parasite densities in patients who later developed treatment failure (geometric mean, 13,450 and 5626 parasites/μL in arms 1 and 2, respectively) were similar to those in patients who were classified as ACPR (8456 and 9219 parasites/μL, *P* = .82 and .49 in arms 1 and 2, respectively).

Parasite clearance times were similar in the 2 groups (*P* = .95). Patients in the azithromycin-artesunate group had a median parasite clearance time of 25.10 h (IQR, 18.50–30.30) and the control group had a median parasite clearance time of 27.15 h (IQR, 18.30–30.20). Patients in the azithromycin-artesunate arm whose treatment failed during the course of the study had longer initial parasite clearance times than those who were cured (median parasite clearance time, 30.7 and 22.2 h, respectively; *P* = .009). At the first assessment in both arms >24
acting antimalarials, treatment response tends to be slow and may possibly be related to a posttreatment prophylactic effect. No difference in the fever clearance times was observed in the 2 groups (P = .59). The median fever clearance times were found to be 6.3 h (IQR, 0.00–19.55) and 3.4 h (IQR, 0.00–19.10) in the azithromycin-artesunate group and in the control group, respectively. When considering only those patients who were febrile on admission, median fever clearance times were 19.3 h (IQR, 16.20–21.53) and 18.5 h (IQR, 6.50–28.15). No difference in gametocyte clearance time was found between the 2 arms (46.1 h vs 97.2 h).

**Safety.** All randomized subjects were analyzed for safety. Significantly more (P = .03) adverse advents occurred in the control group (n = 33) than in the azithromycin-artesunate arm (n = 27). More patients in the control group than in the azithromycin-artesunate arm complained about abdominal pain and discomfort (19.0% vs 7.7%; P = .03). Nausea and vomiting were reported by 2 (6.35%) patients in the control group and by 5 (3.85%) patients in the azithromycin arm (P = .48). Eight patients in the azithromycin-artesunate arm and 2 patients in the control group received a single repeated drug dose after vomiting the study medication within the first hour. During follow-up, upper respiratory tract infections were seen more frequently in the control group than in the azithromycin-artesunate arm, but the difference did not reach significance (9.5% vs 2.3%; P = .06).

Five (3.85%) patients in the azithromycin-artesunate arm complained about loose stool, whereas none of the patients in the control group experienced these symptoms (P = .17). Fatigue, dizziness, and weakness as well as headache occurred in both groups without significant difference. No serious adverse events were reported throughout the study.

**In vitro findings.** A total of 141 samples obtained from all study participants were successfully tested with a histidine-rich protein 2 in vitro drug susceptibility assay for their sensitivity to dihydroartemisinin and azithromycin. Median inhibitory concentrations (IC50) for dihydroartemisinin and azithromycin in parasite samples obtained from patients who later developed treatment failures and those who where cured in the azithromycin-artesunate arm showed no difference (P = .85 and .22, respectively). No correlation (P = .15) was found between parasite clearance time and the IC50 for dihydroartemisinin.

**DISCUSSION**

Azithromycin is a macrolide antibiotic, which has previously shown considerable antimalarial activity in vitro and in vivo when used at adequate doses [3–7]. When azithromycin is given at doses <30 mg/kg per day or not in combination with faster acting antimalarials, treatment response tends to be slow and recrudescence rates are high, particularly in small children [7, 15]. A recent trial conducted in Tanzania using the same drug combination showed that a lower dose of only 20 mg/kg per day of azithromycin resulted in a high number of treatment failures in small African children, emphasizing the need for adequate doses of azithromycin [16]. It is known to be safe in children, and there is extensive experience with its use in pregnant women, the populations most affected by malaria. Azithromycin is available in different formulations and registered in most countries, thereby making it readily available and easy to use.

One of the major advantages of azithromycin over other antibiotics with antimalarial activity is its favorable half-life of ~68 h [17]. Accordingly, drug levels above the minimum inhibitory concentration can be achieved for ~3 life cycles of the parasite with a 3-day regimen, making it a good partner drug for combination with faster-acting antimalarials, such as artemisinin derivatives, which are known to quickly reduce the initial parasite burden. The shorter half-lives commonly found with other antibiotic-based therapies, such as combinations with clindamycin or tetracyclines, typically require the prolongation of treatment, particularly in nonimmune populations and populations in areas with high levels of resistance [18].

In a smaller study previously conducted in Thailand, a group of adult patients who received 750 mg of azithromycin plus 100 mg of artesunate twice daily for 3 days had a cure rate of 92%. In another arm, patients who received a daily dose of 1000 mg of azithromycin plus 200 mg of artesunate over 3 days had a cure rate of 88.9%, which suggests potential dose-dependent activity [2]. Moreover, the respective study demonstrated somewhat longer parasite clearance times, with >70 h in the quinine-azithromycin groups and >30 h in the artesunate-azithromycin groups, which may potentially be related to generally reduced artemisinin sensitivity in Southeast Asia [19].

Because both regimens used in our study largely rely on artemisinin derivatives to quickly reduce the initial parasite burden, parasite clearance times and fever clearance times were similar in both study groups. Patients in the azithromycin-artesunate arm who later had treatment failure during the course of the study needed longer to clear their parasites from the peripheral blood (P = .009). This may be attributable to either interindividual variability of artesunate and dihydroartemisinin blood levels or to a reduced susceptibility to artemisinins. Supporting evidence for lower drug levels in individuals with treatment failure was not available. However, there is no evidence yet of reduced sensitivity to artemisinins in this region [20]. Treatment failure seemed to occur ~1 week later in the control group than in the azithromycin-artesunate arm. This may possibly be related to a posttreatment prophylactic effect.
of lumefantrine, which has a considerably longer half-life than azithromycin.

Compliance with treatment remains a major limiting factor in malaria therapy. Because most of the currently available antimalarial drug combinations require a minimum of 3 days of treatment, a rational concept to improve compliance among outpatients may be the reduction of the total number of doses administered. A 3-dose regimen with azithromycin-artesunate may potentially result in higher effectiveness among outpatients, for whom directly observed therapy is generally difficult to maintain. Compared with twice-daily dosing of antimalarials (as used in our previous studies with the same drug combination), a single daily dose of 1500 mg azithromycin, together with 200 mg artesunate, provides a more convenient dosing schedule and could be expected to lead to higher compliance among outpatients. Previous trials have also shown that a short course of high-dose azithromycin may prove to be an alternative for empirical treatment of patients who present with fever in areas where diagnosis is based on the clinical assessment, as well as for patients with typhoid and other bacterial infections that are frequently found in areas where malaria is prevalent [21, 22]. Intravenous azithromycin combination therapies may also be an interesting option to be explored in the future for patients with severe malaria-like syndromes in areas where concomitant infection or misdiagnosis is common [23].

Our data confirm the safety and tolerability of azithromycin even when given at relatively high doses. Drug-related adverse events were generally rare, and no serious adverse events were reported. Compared with the artemether-lumefantrine control group, a major advantage for the azithromycin-artesunate group seemed to be the significantly lower number of patients complaining about abdominal symptoms. A possible explanation for these findings could be the high rate of lactose intolerance in Asian populations [24–28]. Lumefantrine is highly lipophylic and poorly absorbed when given without fatty food or milk. Giving Coartem with milk improves absorption and tolerance in Asian populations [24–28]. Lumefantrine is highly lipophylic and poorly absorbed when given without fatty food or milk. Giving Coartem with milk improves absorption and tolerance in Asian populations [24–28].

We conclude that treating patients who have uncomplicated falciparum malaria with a 3-dose regimen of azithromycin-artesunate achieves high cure rate with excellent tolerability and may have the advantage of potentially addressing concomitant bacterial infections, which in countries where malaria is endemic are frequently not adequately diagnosed.

Acknowledgments

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References